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### Formation, Characterization, Protein Resistance, and Reactivity of $\text{Cl}_3\text{Si}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OH}$ Self-Assembled Monolayers

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#### ABSTRACT

We report a method for generating tri(ethylene glycol)-terminated-*n*-alkyltrichlorosiloxane monolayers on  $\text{SiO}_2$  surfaces. These chemisorbed films, with a thickness of ~2-3 nm, provide an oligo(ethylene glycol) surface that reduces the nonspecific adsorption of proteins and hydroxyl attachment sites for covalently immobilizing biomolecules to the substrate. These monomolecular films were formed by adsorbing an acetoxy-tri(ethylene glycol)-terminated *n*-alkyltrichlorosilane,  $\text{CH}_3(\text{C}=\text{O})\text{O}(\text{CH}_2\text{CH}_2\text{O})_3(\text{CH}_2)_{11}\text{SiCl}_3$ , onto glass and  $\text{Si}/\text{SiO}_2$  substrates, where the terminal acetate provided a protecting group for the hydroxyl functionality during self-assembly of the film. After formation of the monolayer, the acetate functionality was reduced chemically to form films exposing a covalently attached  $-(\text{OCH}_2\text{CH}_2)_3\text{OH}$  terminus at a density of  $\sim 3 \times 10^{14}$  molecules/ $\text{cm}^2$ . Protein adsorption studies verified that the films exhibited notable resistances against the non-specific adsorption of various proteins. Chemical modification of the  $-(\text{OCH}_2\text{CH}_2)_3\text{OH}$  surface with protein A provided a non-adsorbing surface for selective immobilization of immunoglobulins.

#### INTRODUCTION

The construction of a variety of solid-state devices that interface fabricated systems with biological components or systems requires methods for assembling biomolecules on their surface in controlled ways. Examples include biosensors, chip-based diagnostic assays, and biomaterials used for implants and tissue engineering.<sup>1-4</sup> A key issue in the design of analytical devices that contact biomolecules is that the non-specific adsorption of biological species, particularly proteins, can hinder their performance. For example, the unwanted adsorption of proteins and other species can hinder or alter the selective adsorption of agents to be analyzed, disturb the binding characteristics of immobilized receptor agents used in sensing, and introduce defects in patterned surfaces used in array-based assays. The structural integrity of immobilized biological species are greatly affected by their interactions with the underlying surface, where effects that cause non-specific adsorption can alter their activity and binding abilities as a result of structural changes effected by surface effects. A key need is the ability to have "inert" surfaces that minimize unwanted adsorption events and allow the immobilization of biomolecules.

In a previous paper, we detailed the preparation of self-assembled monolayer coatings by the adsorption of  $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_{2,3}(\text{CH}_2)_{11}\text{SiCl}_3$  onto glass and  $\text{Si}/\text{SiO}_2$  substrates.<sup>5</sup> The resulting chemisorbed films, approximately 2-3 nm in thickness, expressed a densely packed methoxy-capped oligo(ethylene glycol) surface that dramatically retards the non-specific adsorption of various proteins. The methoxy cap provides a chemically inert surface that does not allow subsequent chemical modification as may be wanted for immobilizing a receptor agent onto the surface of a transducer for generation of a biosensor. A target would be a monolayer film that

expressed a terminus that replaced the methoxy cap with a hydroxyl functionality that could be used as a reactive site for subsequent chemical modification.

In this paper, we generate self-assembled monolayers on SiO<sub>2</sub> surfaces that provide the following: (1) a terminal hydroxyl group for possible subsequent on-surface modification, (2) a tri(ethylene glycol) surface for non-fouling characteristics, and (3) an underlying alkyl domain for dense packing within the film. We detail their preparation and characteristics through use as a support for immobilization, here using a binding protein and an immunoglobulin.

## EXPERIMENTAL

### Formation of self-assembled monolayers

Acetyl[(11-trichlorosilyl)undecyl]tri(ethylene glycol) (EG<sub>3</sub>OAc) (**3**) was prepared by the three-step synthesis shown in Figure 1. Silicon wafers were cleaned in 2:8 mixture (v/v) of

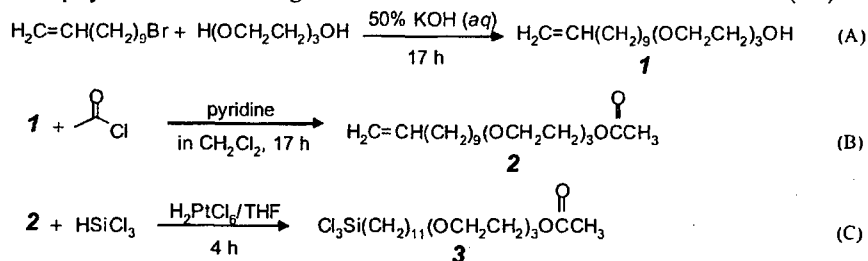


Figure 1. Synthesis of acetyl[(11-trichlorosilyl)undecyl]tri(ethylene glycol) (**3**).

30% H<sub>2</sub>O<sub>2</sub>(aq)/98% H<sub>2</sub>SO<sub>4</sub>(aq) for 10 min, rinsed with distilled water, and blown dried by a N<sub>2</sub> stream. The wafers were immersed into 2-5 mM solution of **3** in anhydrous toluene for 24-48 h, rinsed with toluene, and dried under N<sub>2</sub>. HO-terminated EG<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub> films were formed by immersion of the EG<sub>3</sub>OAc films in LiAlH<sub>4</sub>/ether solutions (see text).

### Protein resistance of EG<sub>3</sub>OH surfaces

Proteins were obtained from Sigma and dissolved in phosphate buffer saline (PBS) solution (10 mM phosphate buffer at pH 7.4, 2.7 mM KCl, and 137 mM NaCl) at 0.25 mg/mL. Samples were immersed in the protein solutions for 24 h, rinsed with deionized water, and dried under N<sub>2</sub>. Amounts of adsorbed protein were determined by ellipsometry using a refractive index of 1.45.

### Subsequent immobilization of biomolecules onto EG<sub>3</sub>OH surfaces

EG<sub>3</sub>OH coated surfaces were immersed in a 0.5 M 1,1-carbonyldiimidazole (CDI) (Aldrich) solution in anhydrous acetonitrile for 1 h, rinsed with acetonitrile, and dried under N<sub>2</sub>. The CDI-reacted surfaces were contacted with a Protein A (Sigma) solution (0.5 mg/mL) in 50 mM carbonate buffer at pH 9.0 at 4 °C for 24 h. The activity of the resulting protein A surfaces were

examined by contacting the surface with a PBS solution containing a model IgG for 30 min and measuring the nitrogen signals from the sample by x-ray photoelectron spectroscopy.

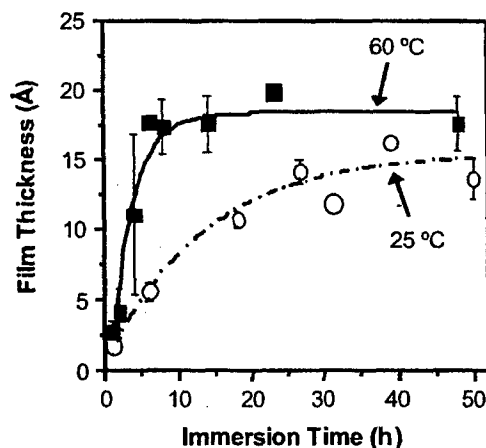
## RESULTS AND DISCUSSION

### Synthesis of the silanating reagent (3)

Acetyl[(11-trichlorosilyl)-undecyl]tri(ethylene glycol) (EG<sub>3</sub>OAc) was prepared by the three-step synthesis shown in Figure 1. Each step was a direct modification of previously reported procedures. The reaction of 11-bromo-undecene and an excess of triethylene glycol and KOH produced **1** in a yield of ~70% as reported previously.<sup>6,7</sup> The hydroxyl group in **1** was protected from reaction with the target -SiCl<sub>3</sub> functionality in the final product by conversion to the acetate **2** (step B). Conversion of **2** to the final silanating product **3** used an excess of HSiCl<sub>3</sub> and H<sub>2</sub>PtCl<sub>6</sub> as a catalyst. Purification of **3** was performed by vacuum distillation.

### Formation of self-assembled monolayers from 3

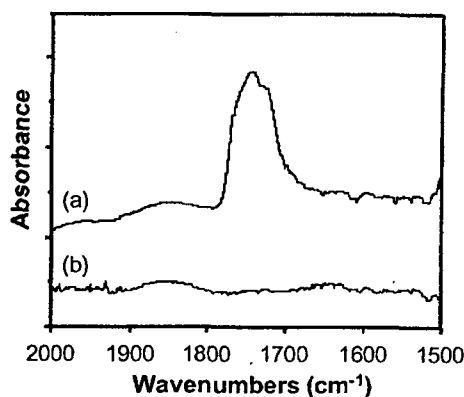
Figure 2 shows thickness data for the formation of EG<sub>3</sub>OAc films onto Si/SiO<sub>2</sub> surfaces at room temperature and 60 °C. At room temperature, the maximum film thickness, as determined ex situ by ellipsometry, was achieved after ~2 days of reaction. At the higher temperature, the reaction achieved its final thickness after only ~8 h, with these conditions generating thicker films than possible by extended reactions at room temperature. The thicker films exhibited lower water contact angles and contact angle hysteresis than the thinner films. The thickest films in Figure 2 (and those presumably the most densely packed) exhibited water contact angles of 70-75° and contact angle hysteresis of ~20°.



**Figure 2.** Kinetics of monolayer formation for reaction of ~4 mM solutions of  $\text{Cl}_3\text{Si}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OC}(=\text{O})\text{CH}_3$  (**3**) in anhydrous toluene onto Si/SiO<sub>2</sub> surfaces.

The terminal acetate groups in monolayer films prepared from **3** were converted to hydroxyl variants by reduction of these surface acetates with  $\text{LiAlH}_4$ . An optimization of reaction conditions determined that the exposure of the acetate-terminated film to 1.0 M  $\text{LiAlH}_4$  in ether for ~10 min at room temperature produced the best results. Under these reaction conditions, the reduction of the acetate caused a decrease in the ellipsometric thickness of the film by ~4 Å, a decrease in the advancing contact angle of water from 70–75° to 62–65°, and evidence for the quantitative conversion of the terminal acetate groups to hydroxyl groups.

Figure 3 shows representative infrared spectra for the film obtained by attenuated total reflection (ATR) for films prepared from **3** before and after reaction with  $\text{LiAlH}_4$ . The spectra were taken on an ATR silicon crystal that had been derivatized with **3**. In Figure 3a, the absorption peak at ~1745  $\text{cm}^{-1}$  corresponds to the stretching band for the ester functionality comprising the terminal acetate group of the  $\text{EG}_3\text{OAc}$  SAM on the ATR crystal. Figure 3(b) shows the same spectral region after reacting the SAM with  $\text{LiAlH}_4$ , and shows a complete loss of the ester peak. Complementary experiments using trifluoroacetic anhydride were performed to confirm the conversion of the terminal acetates to reactive hydroxyl groups by quantification of fluorine signals from the film by x-ray photoelectron spectroscopy (XPS). This analysis determined a site density of  $\sim 3 \times 10^{14}$  molecules/ $\text{cm}^2$  for the  $\text{EG}_3\text{OH}$  surface.

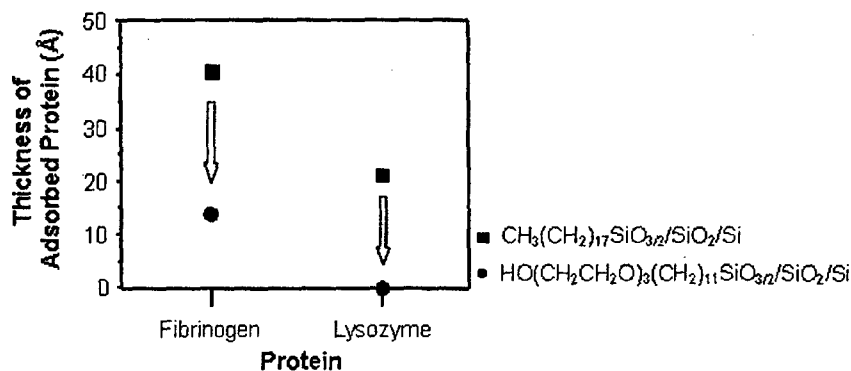


**Figure 3.** ATR/FT-IR spectra of  $\text{Cl}_3\text{Si}(\text{CH}_2)_{11}(\text{OCH}_2\text{CH}_2)_3\text{OC}(=\text{O})\text{CH}_3$  (**3**) SAM on  $\text{Si}/\text{SiO}_2$ . (a) before deprotection and (b) after deprotection.

#### **Protein resistance of $\text{EG}_3\text{OH}$ SAM**

Protein adsorption experiments on the  $\text{EG}_3\text{OH}$ -modified  $\text{SiO}_2$  wafers showed that the coating resulted in significant reductions in the non-specific protein adsorption compared to silanating procedures that hydrophobize the surface by reaction with *n*-octadecyltrichlorosilane [ $\text{CH}_3(\text{CH}_2)_{17}\text{SiCl}_3$ ]. The  $\text{EG}_3\text{OH}$ -terminated films showed no change in film thickness after contact with a 0.25 mg/mL lysozyme solution for 24 h. Similar results were obtained with other proteins, including albumin and insulin. As an example of when the films were not fully able to suppress the non-specific adsorption of a protein, Figure 4 shows data for fibrinogen, where the

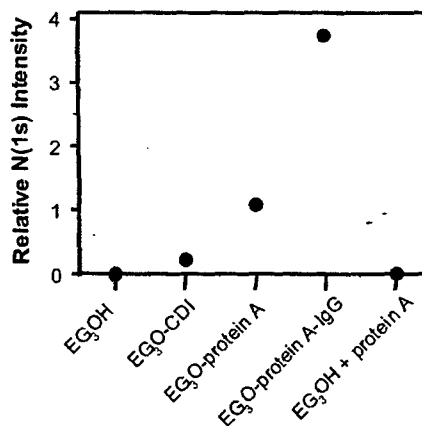
prevention of adsorption was not possible. We note, however, that the adsorbed amount of fibrinogen was reduced by ~70% as compared to the characteristics of hydrophobized octadecyltrichlorosilane (OTS)-treatments (Figure 4).



**Figure 4.** Ellipsometric thicknesses of adsorbed fibrinogen and lysozyme onto EG<sub>3</sub>OH and CH<sub>3</sub> surfaces after contact with 0.25 mg/mL protein solutions for 24 h.

#### Subsequent modification of EG<sub>3</sub>OH SAM surface for immobilization of biomolecules

We used the EG<sub>3</sub>OH surface as a platform for attaching agents to these thin films. Figure 5 shows N(1s) peak intensities taken from XPS spectra of the EG<sub>3</sub>OH surface through its exposure to various agents to develop covalent attachments. We used carbonyl diimidazole (CDI) chemistry to derivatize and activate the hydroxyl-surface for the immobilization of Protein A. In



**Figure 5.** N(1s) peak intensities of the XPS spectra of EG<sub>3</sub>OH surfaces after subsequent sequential modification steps to immobilize Protein A and an IgG. The rightmost data point illustrates the lack of attachment and adsorption of Protein A onto the native EG<sub>3</sub>OH surface.

Figure 5, the initial EG<sub>3</sub>OH surface displayed no N(1s) intensity. Treatment with CDI resulted in a N(1s) signal that increased in intensity when this surface was further treated with Protein A and then a model IgG that becomes bound by the immobilized Protein A. Figure 5 shows that treatment of the EG<sub>3</sub>OH surface with Protein A for 24 h in the absence of activation by CDI results in essentially no attachment or adsorption of Protein A to the surface, in contrast to the results when CDI is employed previously. The suggestion from the data is that the EG<sub>3</sub>OH film presents an "inert" surface towards unwanted non-specific protein adsorption; however, its hydroxyl-termination provides a means for immobilizing proteins and other species to this surface. Together, the EG<sub>3</sub>OH system provides characteristics that offer possibilities as a flexible platform for biosensor construction particularly those such as waveguide technologies that required immobilization and binding events extremely close (a few nanometers) from the transducer surface.

## CONCLUSIONS

The self-assembly of  $\text{CH}_3\text{C}(=\text{O})\text{O}(\text{CH}_2\text{CH}_2\text{O})_3(\text{CH}_2)_{11}\text{SiCl}_3$  (**3**) onto Si/SiO<sub>2</sub> substrates (and presumably onto related oxide surfaces) provides an ability to generate densely packed, hydroxyl-terminated, oligo(ethylene glycol) surfaces. These chemisorbed molecular films exhibit low levels of non-specific protein adsorption and allow subsequent chemical modification for attaching biomolecules onto these hydroxyl-terminated "inert" surfaces. The attachment of Protein A onto this surface and its binding to an immunoglobulin provides new possibilities for immobilizing such species useful in the generation of biosensors and other diagnostic devices.

## ACKNOWLEDGMENTS

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